genes and lethal genes are specifically limited in the claims, and defined in the specification (see pages 11-17, especially pages 11 and 16), to refer to mutually exclusive genes having mutually exclusive effects. As recited in the claims, an essential gene is a gene whose expression is essential to the viability of the cell. A lethal gene is a gene whose expression is lethal to the cell. Thus, a lethal gene according to the claims cannot be an essential gene, and an essential gene cannot be a lethal gene. The expression of the lethal gene and the expression of the essential gene, as recited in the claims, are also mutually exclusive. This distinction between essential genes and lethal genes is important for assessing the relevance of the publications cited in the rejections (see discussion below). A copy of all of the pending claims is attached to this Response in an appendix.

Election of Species

In the Office Action mailed April 15, 1997, the requirement for election of species was maintained based on a standard as recited in MPEP § 806.04(f) which states:

> The general test as to when claims are restricted, respectively, to different species is the fact that one claim recites limitations which *under the disclosure* are found in a first species but not in a second, while a second claim recites limitations *disclosed* only for the second species and not the first. (emphasis added)

Thus, this test requires that the subject matter of claims have **mutually exclusive** subject matter, *as disclosed in the specification*, for restriction to different species. The fact that a limitation *recited* in one claim is not *recited* in another claim that *recites* a different limitation (the situation here) is not enough to make the subject matter of the respective claims distinct species. This can be illustrated using the elected species, a cell having a

regulatory gene down regulating the essential gene (specifically recited in claim 14). The cell of claim 14 (which depends from claim 1 through claim 13) *comprises* an essential gene (recited in claim 1), a lethal gene (recited in claim 1), and a regulatory gene *that regulates the expression of the essential gene* (recited in claim 13) by inhibiting expression of the essential gene (recited in claim 14). Nothing in claims 1, 13, or 14 excludes from the scope of claim 14 a regulatory gene[1] down regulating the lethal gene in a permissive environment ("species" A), a regulatory gene up regulating the lethal gene in a non-permissive environment ("species" B), a regulatory gene down regulating the replication gene in a non-permissive environment ("species" E), a regulatory gene up regulating the replication gene in a permissive environment ("species" F), or an antigen expression gene ("species" G). In fact, the specification makes it clear that the various regulatory schemes of the alleged species can be used together in any combination in a single cell (see, for example, page 10, lines 1-3; and page 18, lines 19-27). It is asserted that, since the cell of claim 14 *encompasses* cells including the various limitations recited in claims representing other alleged "species", and since the disclosure does not indicate in any way that such limitations would not be found in cells having a regulatory gene down regulating the essential gene in a non-permissive environment, it is not proper to restrict the claims to separate species. Accordingly, examination of the full scope of all of the claims is respectfully requested.

[1]It is important to note that such a regulatory gene need not, and preferably is not, the same as the regulatory gene regulating expression of the essential gene. Nothing in the claims or disclosure limits the cells to a single regulatory gene.

Applicants also point out that even were election of species proper for the present claims, all of the claims which encompass the elected species should be examined. In the case of the presently elected species -- cells having a regulatory gene down regulating the essential gene in a non-permissive environment -- the cells of claims 1-14, 16-36, and 38 can all include a regulatory gene down regulating the essential gene in a non-permissive environment. Accordingly, applicants respectfully request that, in the event that the requirement for election of species is maintained, all of claims 1-14, 16-36, and 38 be examined to the extent they read on the elected species.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabling only for the use of some *Salmonella* strains as a vaccine. Applicants respectfully traverse this rejection.

The Office Action notes that many forms of attenuation of *Salmonella* strains reduces their ability to stimulate an immune response (citing Cardenas *et al.*, *Clinical Microbiology Reviews* 5(3):328-342 (1992), and Sigwart *et al.*, *Infection and Immunity* 57(6):1858-1861 (1989)), concluding that such attenuated *Salmonella* strains are unsuitable for use in live vaccines. Based on this, the Office Action concludes that the specification does not enable the use of attenuated *Salmonella* strains as a vaccine.

Applicants initially note that the rejection appears to be based on a misinterpretation of the claimed cells. The object of the claimed Environmentally Limited Viability System is

not attenuated bacterial strains but environmental control of bacterial strains, whether they are attenuated or not. That is, the ELVS is separate from, and is preferably used in combination with, attenuation of the microbial host. The essential gene recited in the claims is *expressed* in the permissive environment (e.g. inside an animal in the case of a vaccine) and so does not contribute to any attenuating effect.

As described in the cited publications, mutations in *asd* and *pur* genes are debilitating to the host cell, going beyond any desirable level of attenuation. It is for exactly this reason that such genes are described throughout the specification as preferred examples of *essential* genes[2] for use in the claimed cells. For the claimed Environmentally Limited Viability System to operate, the lack of expression from an essential gene should render the cell non-viable. Thus, both the *asd* and *pur* genes are clearly identified in the specification as genes involving cell viability and not simple attenuation. In this regard it is also noted that if an *asd* or *pur* mutation is used in an ELVS, an environmentally regulated copy of the *asd* and *pur* gene will be expressed in the permissive environment and the mutation will be complemented. Thus, no detrimental effect as discussed in the Office Action will occur (until and unless the cell is transferred to a non-permissive environment). Accordingly, there is no basis for finding the present claims non-enabled.

The claimed Environmentally Limited Viability System is preferably used in combination with an attenuated microbial host. The use of such hosts is described, for

[2]Essential genes are genes which are required for cell viability (see page 11, lines 15-16).

example, in the paragraph bridging pages 3 and 4, and on pages 33-35 and 41-43 of the specification. These passages indicate that those in the art had identified numerous mutations which could be used to attenuate bacteria. Those in the art were aware, and the specification discusses (see, for example, the sentence bridging pages 34 and 35), the value of insuring that an attenuated strain retain the ability to stimulate an immune response. Thus, the specification makes clear that mutations such as *asd* or *pur* mutations should not be used for attenuation.

Accordingly, applicants submit that the reasoning of the present rejection is unfounded, and that, as a consequence, no *prima facie* case of lack of enablement has been established. For all of the above reasons applicants assert that the claimed cells are fully enabled by the specification.

Rejections Under 35 U.S.C. § 102

Claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120 (1986). Applicants respectfully traverse this rejection.

Gerdes *et al.* (PNAS) disclose (page 3119, second column, and Fig. 3) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene

product which causes rapid cell death. Thus, *hok* can be considered a lethal gene. Gerdes *et al.* (PNAS) fail to disclose any environmentally regulated *essential* gene.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Gerdes *et al.* (PNAS) fail to disclose or suggest such a regulated essential gene, Gerdes *et al.* (PNAS) fail to disclose each and every feature of the claimed cells. Accordingly, Gerdes *et al.* (PNAS) fail to anticipate the claimed cells and method.

Claims 1-4, 10-12, 23, 24, and 27-29 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 4,190,495 to Curtiss, III. Applicants respectfully traverse this rejection.

Curtiss discloses (column 5) *E. coli* having *polA* and *recA* mutations, both alone and in combination. Curtiss discloses a cold-sensitive mutant of *polA* where the polymerase becomes non-functional at low temperatures. Curtiss notes that *recA* mutants are sensitive to ultraviolet light and chemicals and that the combination of the cold-sensitive *polA* mutant and *recA* can lead to rapid degradation of DNA at low temperatures. However, the expression of the *polA* and *recA* mutant genes disclosed by Curtiss are not environmentally regulated. The cold-sensitive *polA* mutant disclosed by Curtiss encodes a cold-sensitive polymerase; the *expression* of the polymerase is not altered to be environmentally regulated. Curtiss also fails to disclose or suggest an environmentally regulated lethal gene.

The claimed cells require **environmentally regulated** expression of a lethal gene and **environmentally regulated** expression of an essential gene. Since Curtiss fails to disclose or suggest such a regulated expression, Curtiss fails to disclose each and every feature of the claimed cells. Accordingly, Curtiss fails to anticipate the claimed cells and method.

Claims 1-3, 8, 10-14, 16, 20, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *EMBO Journal* 5(8):2023-2029 (1986). Applicants respectfully traverse this rejection.

Gerdes *et al.* (EMBO) disclose (page 2024, second column, and Fig. 1) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene. Gerdes *et al.* (EMBO) fail to disclose any environmentally regulated essential gene.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Gerdes *et al.* (EMBO) fail to disclose or suggest such a regulated essential gene, Gerdes *et al.* (EMBO) fail to disclose each and every feature of the claimed cells. Accordingly, Gerdes *et al.* (EMBO) fail to anticipate the claimed cells and method.

Claims 1, 4, 10-14, 20, 23, 24, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Ramos *et al.*, *Bio/Technology* 13:36-37 (1995). Applicants respectfully traverse this rejection.

Ramos *et al.* disclose (page 36, second column) an *E. coli* cell containing a *gef* gene operably linked to P_{lac} which is regulated by LacI (*lac* repressor) expressed from a *lac*I gene operably linked to the *xyl*S gene regulatory elements. The *lac*I gene is expressed in the presence of 3-methylbenzoate (3MB) based on the *xyl*S regulatory elements. When LacI is produced, it represses expression of the *gef* gene. In the absence of 3MB, the *lac*I gene is not expressed and the *gef* gene is derepressed. Expression of the *gef* gene results in Gef-induced cell death. Thus, *gef* can be considered a lethal gene regulated by the presence or absence of 3MB. Ramos *et al.* also disclose (page 36, first column) an *E. coli* cell containing a *hok* gene under control of the *trp* promoter. The *hok* gene is expressed in the absence of tryptophan and not expressed in the presence of tryptophan. Thus, *hok* can be considered a lethal gene regulated by the presence or absence of tryptophan. Ramos *et al.* also disclose (page 37, first column) cells containing a *gef* or *hok* gene operably linked to the randomly switching *fim*Ap promoter. In these cells, the lethal *gef* or *hok* gene is randomly switched on when the promoter inverts. Such regulation is not based on the environment. Ramos *et al.* fail to disclose any environmentally regulated essential gene.

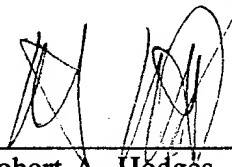
The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Ramos *et al.* fail to disclose or suggest such

a regulated essential gene, Ramos *et al.* fail to disclose each and every feature of the claimed cells. Accordingly, Ramos *et al.* fail to anticipate the claimed cells and method.

Applicants also note that the present rejection is not properly made under 35 U.S.C. § 102(b) since Ramos *et al.* was published less than a year before the present application was filed.

For all of the above reasons, applicants submit that the present claims are patentable. Allowance of claims 1-38 is respectfully solicited.

Respectfully submitted,



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Date: September 15, 1997

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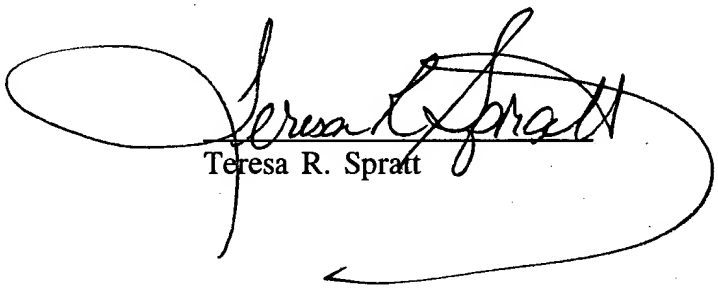

Teresa R. Spratt

Date: September 15, 1997

Appendix

1. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

2. The cell of claim 1 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

3. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

4. The cell of claim 1 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

5. The cell of claim 4 wherein the lethal gene is carried on an extrachromosomal vector and expression of the lethal gene is regulated by an expression product of a regulatory gene.

6. The cell of claim 5 wherein the expression product of the regulatory gene inhibits expression of the lethal gene and is expressed or active only in the permissive environment.

7. The cell of claim 5 wherein the expression product of the regulatory gene induces expression of the lethal gene and is expressed or active only in the non-permissive environment.

8. The cell of claim 4 wherein the vector has two lethal genes.

9. The cell of claim 8 wherein the vector comprises pMEG-104.

10. The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. The cell of claim 1 wherein expression of the essential gene is regulated by an expression product of a regulatory gene.

14. The cell of claim 13 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

15. The cell of claim 13 wherein the expression product of the regulatory gene induces expression of the essential gene and is expressed or active only in the permissive environment.

16. The cell of claim 4 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

17. The cell of claim 16 wherein expression of the replication gene is regulated by an expression product of a regulatory gene.

18. The cell of claim 17 wherein the expression product of the regulatory gene inhibits expression of the replication gene and is expressed or active only in the non-permissive environment.

19. The cell of claim 17 wherein the expression product of the regulatory gene induces expression of the replication gene and is expressed or active only in the permissive environment.

20. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

21. The cell of claim 20 wherein the desired expression product is an antigen.

22. The cell of claim 21 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

23. The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

24. The cell of claim 23 further comprising an expression gene wherein the expression gene encodes a desired expression product.

25. The cell of claim 24 wherein the desired expression product is an antigen.

26. The cell of claim 25 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

27. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment.

28. The method of claim 27 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

29. The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

30. A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

31. The method of claim 30 wherein the system further comprising an expression gene wherein the expression gene encodes an antigen.

32. The method of claim 31 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

33. The method of claim 30 wherein the cell is administered to mucosal surfaces of the animal.

34. The method of claim 33 wherein the mucosal surfaces are in the gastrointestinal tract.

35. The method of claim 30 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for

replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal.

36. The cell of claim 5 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the lethal gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

37. The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

38. The cell of claim 17 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the permissive environment.